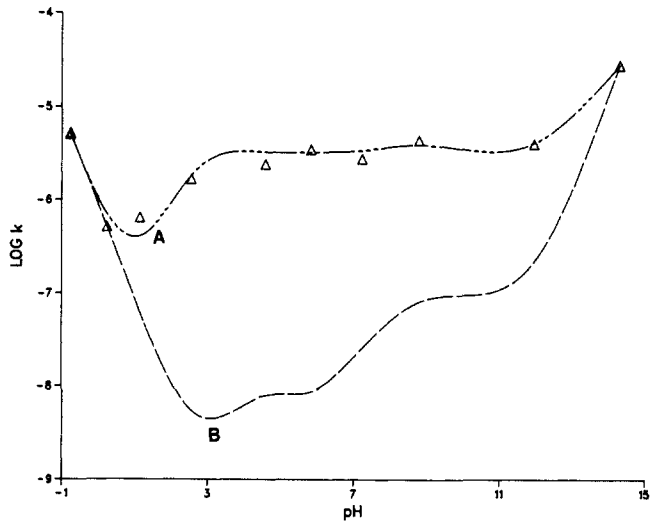


Figure 4. Experimental and calculated racemization rate constants for phenylalanine at various pH values and 142 °C: (Δ) experimental observed rate constants by Bada and Shou;[2] (A) calculated observed rate constants using the six absolute rate constants from this study; and (B) calculated observed rate constants using the four absolute rate constants from Bada and Shou.[2]

Bada and Shou's[2] data points are shown by the Δ's in all three plots. Curve B is the computer-calculated curve using only the four rate constants k_1, k_2, k_4, and k_6 calculated by Bada and Shou.[2] Curve A is the one obtained by using all six rate constants, which follows the experimental points precisely.

The pK_a values used in the calculations of both curves A and B were those obtained in this study using computer analysis. However, there was very little effect on the plots when using pK_a values reported by Bada and Shou[2] or obtained by applying Robinson and Stokes'[4] equations.

Experimental Section

Absolute Rate Constants and pK_a's. The values of different pH's and observed rate constants were entered into the computer. The concentrations of H^+, OH^-, and each ionic species of the amino acids (using the Henderson-Hasselbach equation)[5] were then calculated for each pH. After this, a minimization routine (ZXSSQ from the IMSL Library) using least-squares analysis was called to calculate the best six absolute rate constants. The pK's of the amino acids were determined by manually varying the pK values until the differences between the calculated and experimental values of observed rate constants were minimal.

Observed Rate Constants. The calculated observed rate constants were obtained by entering previously determined pK_a values and absolute rate constants in the computer program and then calculating the resultant observed constants at various values of pH. The pH profile data (Δ points in all plots) are those reported by Bada and Shou.[2] Curve B was obtained by using Bada and Shou's equation (2) with only their four rate constants. Curve A was obtained by using eq 1 with all six rate constants from this study.

Acknowledgment. We thank NASA for research Grant NSG-7038 and the Research Office at Utah State University for computer time.

Registry No. Alanine, 56-41-7; valine, 72-18-4; leucine, 61-90-5; phenylalanine, 63-91-2.

(5) Eisenberg, D.; Crothers, D. *Physical Chemistry with Applications to the Life Sciences*; Benjamin/Cumming: Menlo Park, CA, 1979; p 580.
(6) This is a companion paper to the following paper in this series.

Neighboring Residue Effects: Evidence for Intramolecular Assistance to Racemization or Epimerization of Dipeptide Residues[1]

Grant Gill Smith,* Robert C. Evans, and Rocky Baum

Contribution from the Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322-0300. *Received March 12, 1986*

Abstract: Dipeptides, their methyl esters, diketopiperazines (DKP), and N-substituted derivatives were racemized at high temperatures (approximately 120 °C) in aqueous phosphate buffered solutions at pH values close to the pH of maximum racemization (approximately 8). The racemization of the dipeptides Ala-Gly and Gly-Ala followed reversible first-order kinetics. The initial rate of racemization of DKP was very fast but soon slowed down, supposedly due to hydrolysis. The resulting rate was similar to that of the dipeptides. Esters of dipeptides followed racemization patterns similar to DKP. The racemization rate constants of the dipeptides studied were shown to be independent of the concentration of the dipeptide and the concentration of buffer. A carboxy-terminal proline residue greatly increased the rate of racemization (epimerization) of the amino-terminal residue. Increasing the basicity of the N-terminal amino acid residue increased the rate of racemization (or epimerization) of the C-terminal residue unless the C-terminal residue was sterically hindered as the Ile and Val. Decreasing the basicity of the N-terminal amino acid residue decreased racemization or epimerization for nonhindered C-terminal amino acids. These results support the influence of neighboring groups in the racemization or epimerization of dipeptides. DKP formation is a competing reaction allowing racemization or epimerization in dipeptides. Dipeptide racemization or epimerization is proposed to be the result of a combination of intramolecular base assistance and DKP formation.

Dipeptides racemize or epimerize faster than free or protein-bound amino acids. Some possible explanations are inductive effects, intramolecular assistance, and diketopiperazine (DKP) formation. Each of these factors may have some effect on the racemization (epimerization) of dipeptides. The objective of this research was to study the extent of influence of each factor. With dipeptides, some amino-terminal (N-terminal) amino acids racemize or epimerize faster than carboxy-terminal (C-terminal) amino acids.[2] In others, the reverse is true. Some C-terminal amino acids racemize or epimerize faster than N-terminal ones.[2] This study helps explain why this is observed.

(1) (a) Presented in part at the Fifth IUPAC Conference on Physical Organic Chemistry, Santa Cruz, CA, Aug. 1980; No. B., p 18. (b) Additional data presented at the Utah Academy of Science Arts and Letters, Salt Lake City, UT, April 1984.

(2) (a) Smith, G. G.; de Sol, B. S. *Science (Washington, D.C.)* **1980**, *207*, 765. (b) Kriausakul, N.; Mitterer, R. M. *Science (Washington, D.C.)* **1978**, *201*, 1011. Kriausakul, N.; Mitterer, R. M. *Geochim. Cosmochim. Acta* **1980**, *44*, 753. (c) Steinberg, S.; Bada, J. L. *Science (Washington, D.C.)* **1981**, *213*, 544. (d) Mitterer, R. M.; Kriausakul, N. *Org. Geochem.* **1984**, *7*, 91.

0002-7863/86/1508-7327$01.50/0

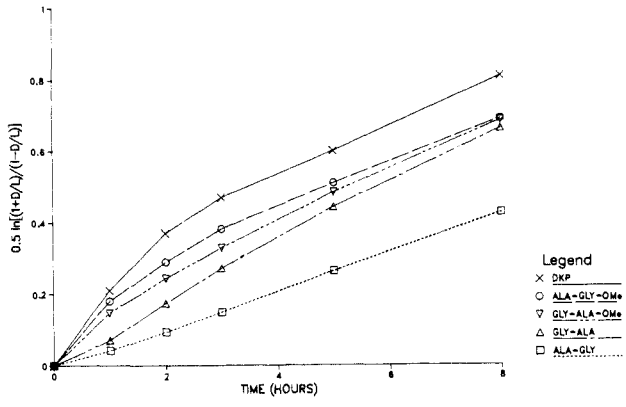


Figure 1. First-order kinetic plot of DKP (X), Ala-Gly-OMe (○), Gly-Ala-OMe (▽), Gly-Ala (△), and Ala-Gly (□) at pH 8.0 and 122 °C.

Racemization (epimerization) of peptide-bound amino acids is a phenomenon of considerable importance both to synthetic peptide chemists[3] and to biogeochemists.[4] The former avoid conditions which might unfavorably alter the desired configuration of the amino acid constituents of peptides during either synthesis or physiochemical analysis, while biogeochemists study the factors affecting racemizaton to effectively apply racemization to geochronology or geothermometry.[4]

Numerous studies have been reported on the racemization of free amino acids[5-7] and amino acid derivatives,[8,9] e.g., esters, amides, and *N*-acetyl derivatives. Racemizations (epimerizations) during amino acid peptide syntheses have also been studied and are reported to occur by DKP formation.[3,9-11]

In this study, dipeptide methyl esters, *N*-methyldipeptides, *N*-acetyldipeptides, acetylamino acids, and DKPs were racemized to compare the rates of racemization (or epimerization) with those of the dipeptides. The samples were racemized (or epimerized) in sealed tubes at high temperature in phosphate buffered solutions at different pH values and at different buffer and peptide concentrations. The amount of racemization was determined by GC analysis of the hydrolyzed, derivatized amino acids with an optically active mixed-phase column.

Dipeptides of the form X-Pro exhibited the most intramolecular assistance with the carboxylate anion of the proline residue assisting in the removal of the methine hydrogen of the X-residue. This was true even when X was a sterically hindered residue. Intramolecular base assistance from the amino group of the N-terminal of dipeptides was also important. When either assistance mechanism was prevented by estrification or retarded by steric factors, DKP formation was the preferred route of racemization. Intramolecular assistance and DKP formation were diminished for C-terminal amino acids when the amino group of the N-terminal amino acid was made less basic by acetylation or located further from the reaction site. Increasing the basicity of the N-terminal nitrogen by introducing an *N*-methyl group increased the racemization rates of nonsterically hindered C-terminal residues. This added steric effect decreased the rates of the already sterically hindered residues as in Gly-Val or Gly-Ile. It appears that steric hindrance affects both DKP formation and intramolecular assistance.

Table I. Comparison of the Racemization or Epimerization of Amino Acids in Dipeptide, Dipeptide Methyl Esters, and Diketopiperazines

case	compd	pH[a]	*T*, °C (±0.5)	time,[b] h	% D isomer[c]
I	Ala-Pro[d]	7.6	120	4.0	8.7
	Ala-Pro-OMe				4.9
	DKP				9.1
	Val-Pro	8.0	122	8.0	33.7
	Val-Pro-OMe				11.4
	DKP				21.2
	Ile-Pro	8.0	120	8.0	29.5
	Ile-Pro-OMe				6.1
	DKP				22.9
II	Ala-Gly	8.0	122	8.0	28.7
	Ala-Gly-OMe				37.4
	DKP				41.0
	Val-Gly	8.0	120	8.0	11.9
	Val-Gly-OMe				18.2
	DKP				29.7
	Ile-Gly	8.0	120	12.0	21.3
	Ile-Gly-OMe				35.8
	DKP				43.8
III	Gly-Ala	8.0	122	8.0	36.7
	Gly-Ala-OMe				37.3
	DKP				41.0
	Gly-Val	8.0	120	8.0	3.6
	Gly-Val-OMe				11.7
	DKP				29.7
	Gly-Ile	8.0	120	12.0	6.4
	Gly-Ile-OMe				32.6
	DKP				43.8

[a] pH values at 25 °C. [b] Note change in reaction time. [c] Average of three separate racemization or epimerization studies. [d] Analysis for isomers of proline was not practical with the chiral phase employed.

Results and Discussion

First-Order Kinetics. The racemization of dipeptides (Ala-Gly and Gly-Ala) follows reversible first-order kinetics and showed different observed rate constants (1.5×10^{-5} and 2.3×10^{-5} s^{-1}, respectively, at 122 °C and pH 8.0, Figure 1). The rate constants were independent of peptide concentration, indicating that there is no intermolecular reaction between dipeptide molecules. Racemization of these dipeptides is approximately 100 times faster than for free Ala (3.2×10^{-7} s^{-1}). The rate constant for racemization of Ala at the carboxy-terminal position (Gly-Ala) is 1.53 times larger than it is when it is located at the amino-terminal position (Ala-Gly). This confirms Smith and Sol's observation where only amounts of racemization (percent D form) were reported.[2] The much faster rate for dipeptides and the fact that the two dipeptides do not racemize at the same rate suggests that there is a significant intramolecular effect.

Esters and DKP Racemization (or Epimerization) Studies. To investigate the nature of this intramolecular effect, the racemization rates of the methyl esters of two dipeptides (Gly-Ala-OMe and Ala-Gly-OMe) and their DKP were studied, Figure 1. The initial rates of racemization (or epimerization) for the esters and the DKP are faster than those for the dipeptides. It is well-known that dipeptide esters more easily cyclize to diketopiperazine than do the dipeptides.[3,12] Apparently, the esters cyclize to form the rapidly racemizing (or epimerizing) DKP, hydrolysis of the DKP

(3) (a) Kemp, D. S. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1979; Vol. 1, pp 315–383. (b) Kovacs, J. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1980; Vol. 2, Part A, pp 486–567.

(4) For reviews of geochronological applications of amino acid racemization, see: (a) Kvenvolden, K. A. *Annu. Rev. Earth Planet. Sci.* **1975**, *3*, 183. (b) Schoreder, R. A.; Bada, J. L. *Earth Sci. Rev.* **1976**, *12*, 347. (c) Williams, K. M.; Smith, G. G. *Origins Life* **1977**, *8*, 91. (d) Davies, W. D.; Treloar, F. E. *Artefact* **1977**, *2*, 63. (e) Rutter, N. W.; Crawford, R. J.; Hamilton, R. D. *Geosci. Canada* **1979**, *6*, 122. (f) *Biogeochemistry of Amino Acids*; Hare, P. E., Hoering, T. C., King, K., Jr., Eds.; Wiley: New York, 1980. (g) Bada, J. L. *Annu. Rev. Earth Planet. Sci.* **1985**, *13*, 241.

(5) Bada, J. L. *J. Am. Chem. Soc.* **1972**, *95*, 1371.

(6) (a) Smith, G. G.; Williams, K. M.; Wonnacott, D. M. *J. Org. Chem.* **1978**, *43*, 1. (b) Bada, J. L.; Shou, M. Y. ref 4f, pp 235–253. (c) Smith, G. G.; Evans, R. C. ref 4f, pp 267–282.

(7) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627.

(8) (a) Matsuo, H. Y.; Kawazoe, Y.; Sato, M.; Ohnishi, M.; Tatsuno, T. *Chem. Pharm. Bull.* **1967**, *15*, 391. (b) Matsuo, H. Y.; Kawazoe, Y.; Sato, M.; Ohnishi, M.; Tatsuno, T. *Chem. Pharm. Bull.* **1970**, *18*, 1788. (c) Sato, M.; Tatsuno, T.; Matsuo, H. *Chem. Pharm. Bull.* **1970**, *18*, 1974.

(9) Kovacs, J.; Mayers, G. L.; Johnson, R. H.; Cover, R. E.; Chatak, U. R. G. *J. Org. Chem.* **1970**, *35*, 1810. (b) Kovacs, J.; Cortegiano, H.; Cover, R. E.; Mayers, G. L. *J. Am. Chem. Soc.* **1971**, *93*, 1541. (c) Kovacs, J.; Holleran, E. M.; Hui, K. Y. *J. Org. Chem.* **1980**, *45*, 1060.

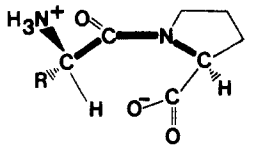
(10) (a) Goodman, M.; Levine, L. *J. Am. Chem. Soc.* **1964**, *86*, 2918. (b) Goodman, M.; McGahren, W. J. *J. Am. Chem. Soc.* **1965**, *87*, 3028.

(11) Benoiton, N. L.; Kuroda, K.; Cheung, S. T.; Chen, F. M. F. *Can. J. Biochem.* **1979**, *57*, 776.

(12) Purdie, J. E.; Benoiton, N. L. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1845.

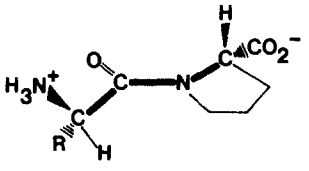
occurs to form a mixture of the dipeptides, and the observed rate constants of the esters and DKP soon parallel those of the dipeptides.

X-Pro Dipeptides, Case I, Table I. A comparison of the extent of racemization or epimerization of amino acids in dipeptides, dipeptide esters, and DKP is shown in Table I. The most striking results are seen with Ala-Pro, Val-Pro, Ile-Pro, their methyl esters, and their DKP. The free dipeptide, Val-Pro, racemizes 4 times faster than its methyl ester, Val-Pro-OMe, and 2 times faster than its DKP. (Rate ratios are based on rate constants which are calculated by using the equation $k = \ln [(1 + \text{D/L})/(1 - \text{D/L})]/2t$ for reversible first-order kinetics.) A similar result was found for Ile-Pro epimerization and Ala-Pro racemization (Table I, case I). The fact that the peptide racemizes faster than its methyl ester or DKP is strong evidence that the DKP is not an important intermediate in the epimerization of dipeptides of the type X-Pro. The free carboxylate anion of the proline residue apparently acts as an intramolecular base to assist in the removal of the methine hydrogen. The carboxylate anion is particularly active in X-Pro dipeptides because the peptide linkage with Pro dipeptides is largely in the cis configuration.[2] Blout et al.[13] showed that Pro, Ile, Val, and other similar amino acids (sarcosine, hydroxyproline, and threonine) caused peptides to form the nonhelical structure. Because of the partial double-bond character of the C–N bond, the four atoms of the peptide linkage plus the two adjacent α-carbon atoms lie in the same plane, making possible cis and trans isomers. Generally speaking, *trans*-peptides are favored. With X-Pro dipeptides the linkage can be either cis (**1**) or trans (**2**).[14] Dorman and Bovey[15] and Wurthrich et al.[16] reported that the negative carboxylate in H^+X-Pro favored the cis form. Therefore, at the pH of this study (7–8) we expect, and apparently observe, considerable carboxylate intramolecular assistance in the racemization (epimerization) of X-Pro dipeptides.



cis-X-PRO

1



trans-X-PRO

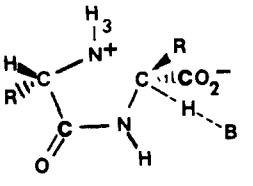
2

X-Gly Dipeptides, Case II, Table I. In case II, we consider those dipeptides where Gly is at the carboxyl position (Ala-Gly, Val-Gly, and Ile-Gly). With these three dipeptides the esters react faster than the dipeptides, but they all react slower than their DKP. Intramolecular assistance by the carboxylate group is not expected to be as much with these dipeptides as with X-Pro dipeptides for the reasons discussed above. If DKP formation is the preferred reactive intermediate, we expect the rates of the esters to be much faster than those of the dipeptides and the rates close to that for the DKP. Perhaps DKP formation [not racemization (epimerization)] is the rate-determining step with these compounds. Certainly some type of intramolecular interaction must be occurring because the reaction rates for these dipeptides are much faster that those for the free amino acids.[2]

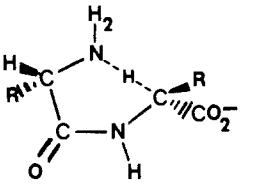
Steinberg and Bada,[17] in a very careful study on the epimerization of Gly-Ile and Ile-Gly, clearly showed that each dipeptide forms a DKP and each is inverted to some extent to the other dipeptide. The extent of inversion and epimerization that takes place is of particular interest. During the epimerization of Ile-Gly at pH 8.66 for 12 h, they report that only 3% of it was inverted to its isomeric dipeptide (Gly-Ile). They found that the DKP, which was isolated in 12% yield, was completely epimerized (allo-Ile/Ile = 1.4, the equilibrium value), but the newly formed Gly-Ile was epimerized only slightly (allo-Ile/Ile = 0.20). The original dipeptide, Ile-Gly, was also only slightly epimerized (allo-Ile/Ile = 0.16). These results suggest that DKP, even though it is formed under these reaction conditions from Ile-Gly, is not necessarily the only intermediate in epimerization. It appears that Ile-Gly does not readily form a DKP. We propose that the intramolecular effect in Ile-Gly is the carboxylate anion serving as an intramolecular base in removing the methine hydrogen on the amino-terminal residue similar to the case for Ile-Pro only not as much.

Gly-X Dipeptides, Case III, Table I. The electrostatic theory predicts that the N-terminal residue of dipeptides should racemize faster than the C-terminal. The positive charge on the nitrogen should activate and the negative charge on the carboxylate should retard racemization. To explain why some C-terminal amino acids racemize faster, Smith and Sol[2] suggested that the N-terminal group assisted the racemization at the C-terminal through an intramolecular effect.

The neighboring NH_3^+ group could stabilize the incipient carbanion generated at the C-terminus (ion–dipole stabilization) (**3**), or the neighboring NH_2 group could assist in the removal of the α-hydrogen at the C-terminus by acting as an intramolecular base which has a very favorable entropy term (**4**). With Gly-X



3



4

dipeptides, several facts are readily apparent. The dipeptide Gly-Ala reacts appreciably faster than Gly-Val and Gly-Ile, which are hindered dipeptides (note that with Gly-Ile the reaction time is 12 h, Table I). With Gly-Ala, the dipeptide racemizes at approximately the same rate as its ester (36.7% D vs. 37.3% D), but with the sterically hindered dipeptides, Gly-Val and Gly-Ile, their esters react faster than the dipeptides (3.6% D vs. 11.7% D; 6.4% D vs. 32.6% D). Finally, the DKPs of Gly-Val and Gly-Ile react appreciably faster (12–15 times) than either the dipeptides themselves or the methyl esters (2–3 times). Here it is reasonable to conclude that if DKP is the reactive intermediate, its formation is rate determining, not its racemization (epimerization). We present two logical explanations why esters of Gly-Val and Gly-Ile react faster than the free dipeptides.[18]

Esters of amino acids are more reactive in racemization (epimerization) than are the free amino acids.[8,9] One explanation for this is that the negative charge on the carboxylate anion, which electrostatically reduces the acidity of the α-methine carbon in

(13) Blout, E. R.; Schmeir, L.; Simmons, N. S. *J. Am. Chem. Soc.* **1962**, *84*, 3193.
(14) Patel, D. J. *Biochemistry* **1973**, *12*, 667.
(15) Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.
(16) See ref 4–30 of: Wuthrich, K.; Grathwohl, B.; Schwyzer, R. *Peptides, Polypeptides and Proteins*; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; Wiley: New York, 1974; p 300.
(17) Steinberg, S.; Bada, J. L. *Science (Washington, D.C.)* **1981**, *213*, 544.

(18) This is a companion paper to the preceding paper in this issue. The preceding paper describes the need for considering six absolute rate constants when comparing rate constants vs. pH. These six rate constants deal with the acid- and base-catalyzed reactions for the three forms of the amino acids (+, 0; +, –; and 0, –). Theoretically, dipeptides might be treated in a similar fashion as amino acids if they did not involve many other reactions. Under racemization or epimerization conditions, dipeptides also cyclize to form diketopiperazines which react very rapidly, and this reaction could also be acid and base catalyzed. Dipeptides also are involved in a variety of intramolecular reactions. Because of all these reactions, it is not practical to treat the problem in the same manner as it was treated for amino acids. However, this does not alter the fact that factors other than inductive effects from the carboxyl, amine hydrochloride, and/or the R group affect the racemization (epimerization) of dipeptides. In this paper the observed rate constants for all reacting species are reported. Using only the combined rate, however, does not alter the findings. These combined rate constants demonstrate that the carboxylate anion, the amino group, and possibly the amine hydrochloride group influence the observed rate through intramolecular effects. These intramolecular effects are similar to many other intramolecular effects found in chemistry which occur because of the favorable entropy factor.

Table II. Effect of Altering the Basicity of Neighboring Groups on the Rate of Racemization of the C-Terminal Amino Acid at 122 °C, pH 8[a]

compd	R	A dipeptide[c] N-terminal X-Gly	B dipeptide[d] C-terminal Gly-X	C *N*-methyldipeptide[e]	D *N*-acetyldipeptide[f]	E β-alanyldipeptide[g]	F acetylamino acid[h]
1	Me	24.6	33.3	42.8	2.7	2.7	1.5
2	*i*-Bu	20.3[b]	26.1	30.0	5.0	2.0	2.7
3	benzyl	22.5	33.1	37.2	3.5	1.8	1.0
4	*i*-Pr	13.0[b]	5.6	1.7		0.8	1.1
5	*sec*-Bu	16.5	4.3	1.5		1.6	1.1

[a] Values given as percent D isomer. [b] Smith and de Sol, ref 2a. [c] $H_3N^+C(R)HCONHCH_2CO_2^-$. [d] $H_3N^+CH_2CONHC(R)HCO_2^-$. [e] $CH_3NH_2^+$-$CH_2CONHC(R)HCO_2^-$. [f] $CH_3CONHCH_2CONHC(R)HCO_2^-$. [g] $H_3N^+CH_2CH_2CONHC(R)HCO_2^-$. [h] $HCH_2CONHC(R)HCO_2^-$.

the free acid, is removed in the ester. The same argument can be involved to explain the observation that Gly-Val-OMe racemizes 3 times faster than its dipeptides. Likewise, Gly-Ile-OMe epimerizes 5 times faster than its dipeptide (Table I, case III). A second logical explanation is that DKP formation occurs several times faster from the ester than it does from the free dipeptides.

Steinberg and Bada[17] report that heating Gly-Ile for 48 h at 131 °C and pH 8.66 causes 30% of it to invert to Ile-Gly, and all of the Ile in Ile-Gly is epimerized (allo-Ile/Ile = 1.4). Only a very small amount was isolated as DKP (0.01%), and it too was completely epimerized. The original dipeptide, Gly-Ile, epimerized very slightly (allo-Ile/Ile = 0.03). Therefore, with Gly-Ile we conclude that the small portion that forms a DKP will quickly epimerize and subsequently hydrolyze primarily to Ile-Gly but not to Gly-Ile. Had hydrolysis of the highly reactive DKP taken place to form Gly-Ile, more of the Gly-Ile would have been epimerized. The results reported in this study on Gly-Ile support Steinberg and Bada's[17] findings and also the earlier work by Smith and Sol.[2] Dipeptides of hindered amino acids (Val and Ile) form a special class. Intramolecular base assistance by the amino group is not as evident in racemization (epimerization) as it is with less-hindered dipeptides (Gly-Ala, Gly-Leu, and Gly-Phe). As a consequence, very little racemization occurs with Gly-Val and little epimerization with Gly-Ile. Esterification of these dipeptides promotes DKP formation.[3,12] The esters may racemize by forming a DKP intermediate. However, there is evidence that these hindered dipeptides (Gly-X) do not react entirely via a DKP intermediate (see Results and Discussion of the preceding paper in this issue).

Neighboring-Group Assistance in Gly-X Dipeptides. To further study the intramolecular base effect from the N-terminal residue on the C-terminal residue, modifications were made in the dipeptide Gly-X to alter the base strength of the nitrogen (Table II). A methyl group attached to nitrogen increases the basic strength, and in turn racemization rate (column C vs. column B, Table II, and acetylation of the nitrogen markedly reduces its basic strength which reduces the racemization rate (column D vs. column B, Table II). The results for these derivatives are very striking. N-Methylation of the nonsterically hindered residues (Ala, Leu, and Phe) causes racemization to take place faster (Sar-X vs Gly-X). The effect is greatest for the least hindered residue, Ala. With the two sterically hindered residues (Val and Ile), methylation reduces racemization (epimerization). Therefore, when steric hindrance is not critical the more basic amine (Sar-X) enhances intramolecular removal of the methine hydrogen on the carboxy-terminal residue. The fact that the rate is so much slower for the highly sterically hindered dipeptides (Gly-Val and Gly-Ile) than the less sterically hindered ones (Gly-Ala, Gly-Leu, and Gly-Phe) suggests that steric hindrance may actually cause a change in the mechanism. The mechanism for racemization (epimerization) for these sterically hindered dipeptides, as previously stated, occurs primarily via the DKP intermediate as Steinberg and Bada[17] have suggested for Gly-Ile. From both this study and the Steinberg and Bada[17] study we conclude that intramolecular base effects are more prominent when steric interaction is not critical, and there is a critical balance between steric and electronic effects.

Substituting an acetyl group on the amino end of Gly-X markedly reduces the rates to approximately one-tenth (column B vs. column D, Table II). In addition to reducing the basicity of the amino group, it prevents DKP formation, and the acetyl group also increases the incipient steric interaction. The combination (markedly) reduces the racemization at the C-terminals. Acetylation would not affect racemization at the C-terminal position by induction through the σ-bonds.

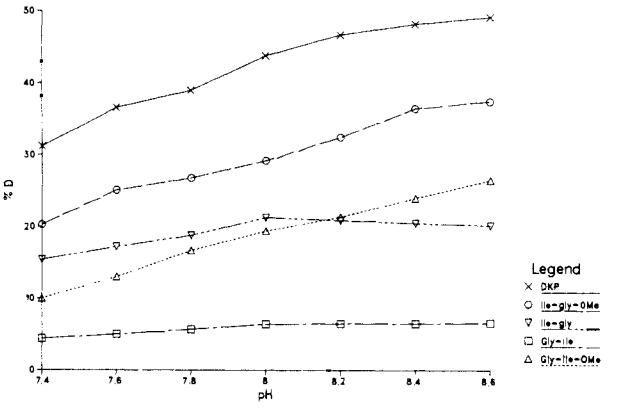


Figure 2. Effect of pH on the racemization of DKP (X), Ile-Gly-OMe (○), Ile-Gly (▽), Gly-Ile-OMe (△), and Gly-Ile (□) at 120 °C for 12 h.

When the amino group is placed one carbon further away as in β-alanyl-X, a seven-membered ring is necessary for any intramolecular amino assistance and/or formation of a cyclic DKP-like intermediate. With these dipeptides there appears to be very little, if any, intramolecular interaction with the amino-terminal as observed in the marked reduction of racemization (column E vs. column B, Table II) at the C-terminal position.

Of course, complete removal of the amino group precludes any intramolecular assistance or DKP formation and would dramatically reduce the extent of racemization for all amino acids. This was observed (column F, Table II). These compounds are not dipeptides but rather acetylamino acids.

The values for X-Gly are given in column A of Table II to illustrate that carboxy-terminal amino acids react faster for nonsterically hindered amino acids, but the amino-terminal ones react faster for the sterically hindered ones (Val and Ile).

Effect of pH on Epimerization. Figure 2 shows how pH influences epimerization of Gly-Ile, Ile-Gly, their methyl esters, and their DKP. Between pH 7.8–8.6 the rate increases with pH for the esters and DKP. With the dipeptides, inflection points occur at about pH 8. Above pH 8 the reactivity is decreased for Ile-Gly and Gly-Ile. Steinberg and Bada[17] also found a decrease in the epimerization of Ile-Gly and Gly-Ile above pH 8 but much more pronounced. It is doubtful that these dipeptides and their esters could epimerize via the same mechanism and show such contrasting pH profiles. We propose that these esters cyclize to the DKP prior to epimerizing. The dipeptides may epimerize via intramolecular assistance involving either the carboxylate anion, **5** and **6**, or the amino group, **3** and **4**. The reason for the inflection points at pH 8 for Ile-Gly and Gly-Ile could be due to a reduction of the zwitterion concentrations. The protonated amine **3** appears to enhance epimerization of Gly-Ile to a greater extent than the deprotonated form **4** because epimerization decreases at higher values of pH even though the concentration of hydroxide catalyst

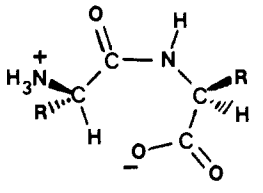
Table III. Effect of Peptide Concentration on the Observed Racemization Rate Constants of Ala-Gly, DKP, and Gly-Ala at pH 8.0 and 120 °C for 8 h

peptide concn, M	Ala-Gly		DKP		Gly-Ala	
	% D	k, h^{-1}	% D	k, h^{-1}	% D	k, h^{-1}
0.001	23.2	0.039	39.9	0.100	32.1	0.064
0.010	23.3	0.039	37.9	0.089	32.3	0.065
0.020	23.2	0.039	36.6	0.082	31.0	0.061

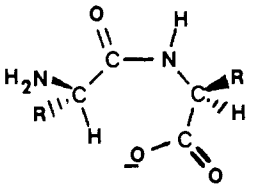
Table IV. Effect of Buffer Concentration on the Observed Racemization Rate Constants of Ala-Gly, DKP, and Gly-Ala at pH 8.0 and 120 °C for 8 h

buffer concn, M	Ala-Gly		DKP		Gly-Ala	
	% D	k, h^{-1}	% D	k, h^{-1}	% D	k, h^{-1}
0.025	22.9	0.038	31.5	0.091	38.3	0.062
0.050	23.9	0.041	32.0	0.094	38.8	0.064
0.100	25.0	0.043	32.4	0.095	39.0	0.065

increases. The protonated form of the amino group enhances epimerization of Ile-Gly by inductively reducing the charge on the incipient carbanion formed during epimerization (**5**). As the pH increases, the concentration of the deprotonated form increases (**6**).



5



6

Ile-Gly-OMe has no carboxylate anion and cannot show the type of intramolecular effect as shown in **5** and **6**. The inflection points for Gly-Ile and Ile-Gly are not thought to be due to peptide hydrolysis at pH 8 because peptide hydrolysis requires more drastic conditions, and the esters and DKP are not showing any inflection.

Steinberg and Bada have shown thermodynamic equilibrium to favor Ile-Gly over Gly-Ile during hydrolysis reactions of DKP.[17] This indicates that Ile-Gly has less tendency to form the DKP than does Gly-Ile, and yet Ile-Gly epimerizes 4.1 times as fast (Table I). We conclude that Gly-Ile and Ile-Gly epimerize (Gly-Val and Val-Gly racemize) via *both* DKP formation and intramolecular base assistance at pH 8 and that any sterically hindered Gly-X and X-Gly will behave similarly.

The buffer concentration study (Table IV) verified that racemization was essentially independent of buffer concentration. The fact that the rate of racemization is essentially independent of buffer concentration (Table IV) and peptide concentration (Table III) strongly supports the lack of general-acid catalysis and bimolecular reactions. As the pH is changed the concentration of the zwitterion changes, attenuating the effect by hydroxide ion concentrations. These results, coupled with the kinetic study, give credence to the importance of an intramolecular base-catalyzed mechanism in the racemization of dipeptides. X-Pro dipeptides show the greatest amount of intramolecular assistance for racemization (Table I).

Peptide and Buffer Concentration Study. When the concentration of dipeptides is varied, esters and DKP (Table III) show that peptide molecules do not act as intermolecular bases. The ratio of D isomer for Gly-Ala and Ala-Gly did not change as the concentration was increased by 20 times. This was also true for Ala-Gly-OMe. The rate actually decreases slightly for Gly-Ala-OMe and the DKP.

Conclusions

Of the dipeptides studied, only Gly-Val and Gly-Ile show evidence of racemizing (epimerizing) primarily via a DKP intermediate in the pH range of optimum racemization. The formation of DKP, although it was thought to be a general reaction intermediate,[17] is only a competing reaction. The strongest evidence for intramolecular assistance to racemization is seen with dipeptides of the form X-Pro. For dipeptides with nonsterically hindered amino acids, e.g., Gly-Ala and Ala-Gly, the C-terminal racemizes faster than the N-terminal. This is attributed to an intramolecular effect. In a study of the racemization of esters of the type Ile-Gly-OMe and Gly-Ile-OMe, DKP is a likely reactive intermediate.

Experimental Section

Dipeptide Analogues. The dipeptide methyl esters were made from (*tert*-butyloxy)carbonyl-X (Boc-X) and Y-OMe amino acid compounds and coupled by *N*,*N*-dicyclohexylcarbodiimide (DCC) coupling in CH_2Cl_2. The Boc-X-Y-OMe compounds were purified by column chromatography using 230–400-mesh silica gel and 7:3 hexane/acetone eluent. The Boc protecting group was removed by adding a saturated HCl/CH_2Cl_2 solution at 0 °C and allowing it to react at room temperature overnight. The methyl ester hydrochloride salts were purified by precipitation.

The DKPs were made from the dipeptide methyl esters by deprotonating the HCl salts of the esters with saturated aqueous sodium bicarbonate solution, evaporating the water under a stream of air at room temperature, extracting with acetone, and refluxing the esters in methanol for a minimum of 24 h (there was less than 1% racemization for all DKPs). The esters and DKPs were characterized by melting points and NMR.

The dipeptides, their *N*-methyl and *N*-acetyl derivatives, the alanyldipeptides, and *N*-acetylamino acids were purchased from Vega Biochemicals, Tucson, AZ.

Melting points were determined on a Thomas-Hoover capillary melting point apparatus. ^1H nuclear magnetic resonance spectra were recorded on a Varian EM 360 and/or a JEOL FX 90Q FT spectrometer.

Val-Pro-OMe·HCl: NMR δ 1.2 (d, 6), 2.0 (m, 3), 2.3 (m, 2), 3.5 (m, 1), 3.7 (s, 3), 4.0 (m, 1), 4.3 (m, 1), 4.6 (m, 1), 8.3 (br s, 3); mp 145–148 °C.

Val-Pro DKP: NMR δ 1.0 (2d, 6), 2.1 (m, 5), 3.4–4.2 (m, 3), 7.3 (d, 1); mp 193–196 °C.

Gly-Val-OMe·HCl: NMR δ 1.0 (d, 6), 2.2 (m, 1), 3.7 (s, 3), 4.3 (m, 3), 8.4 (br s, 3), 8.9 (br d, 1); mp 81–83 °C.

Gly-Val DKP: NMR δ 1.31 (d, 6), 2.5 (m, 1), 3.7 (d, 1), 3.8 (m, 1), 8.1 (br m, 2); mp 256–258 °C [lit.[19] mp 264–265 °C].

Val-Gly-OMe·HCl: NMR δ 1.2 (d, 6), 2.3 (m, 1), 3.7 (s, 3), 3.9 (m, 3), 8.5 (br m, 3), 9.3 (br t, 1); mp 67–68 °C.

Ala-Pro-OMe·HCl: NMR δ 1.6 (d, 3), 2.1 (m, 4), 3.7 (s, 3), 3.7 (t, 2), 4.3 (br d, 1), 4.6 (t, 1), 8.5 (br s, 3); mp 120–122 °C.

Ala-Pro DKP: NMR δ 1.0 (d, 3), 1.7 (m, 4), 3.4 (m, 2), 4.2 (m, 2), 7.3 (d, 1); mp 173–175 °C.

Ile-Pro-OMe·HCl: NMR δ 1.1 (m, 8), 2.0 (m, 5), 3.6 (t, 2), 3.7 (s, 3), 4.0 (m, 1), 4.6 (m, 1), 8.2 (br s, 3); mp 70–72 °C.

Ile-Pro DKP: NMR δ 1.0 (m, 8), 2.0 (m, 5), 3.6 (m, 4), 7.3 (d, 1); mp 104–106 °C.

Gly-Ala-OMe·HCl: NMR δ 1.2 (d, 3), 3.7 (m, 5), 4.2 (m, 1), 8.0 (br s, 3), 9.0 (d, 1); mp 160–162 °C [lit.[12] mp 160–160.8 °C].

Gly-Ala DKP: NMR δ 1.3 (d, 3), 3.7 (m, 3), 7.9 (br s, 1), 8.1 (br s, 1); mp 238–240 °C [lit.[20] mp 228–230 °C].

Ala-Gly-OMe·HCl: NMR δ 1.4 (d, 3), 3.7 (s, 3), 3.9 (m, 3), 8.4 (br s, 3), 9.1 (br t, 1); mp 158–160 °C [lit.[12] mp 161–162.5 °C].

Gly-Ile-OMe·HCl: NMR δ 1.0 (d, 6), 1.3 (m, 2), 1.8 (m, 1), 3.7 (s, 3), 4.5 (m, 3), 8.4 (br s, 3), 8.9 (br d, 1); mp 60–62 °C.

Gly-Ile DKP: NMR δ 0.9 (t, 6), 1.1 (m, 2), 1.9 (m, 1), 3.7 (m, 3), 8.0 (m, 2); mp 258–260 °C.

Ile-Gly-OMe·HCl: NMR δ 1.1 (m, 6), 1.4–1.9 (br m, 3), 3.7 (s, 3), 4.2 (m, 1), 4.5 (m, 2), 8.3 (br s, 3), 8.8 (br s, 1); mp 180–181 °C.

Temperature Control. The temperature of the samples during racemization was controlled to ±0.5 °C in a thermostatically controlled bath of heavy-duty synthetic aircraft oil. The dipeptide, its methyl ester, and its DKP were all racemized under the same conditions and at the same time in a high-temperature bath. The bath was regulated via a proportional temperature controller (RFL Industries, Inc., Model 70A-115) and calibrated against a National Bureau of Standards calibrated platinum resistance thermometer.

Sample Preparation. The dipeptide, dipeptide methyl ester, and diketopiperazine aqueous solutions were prepared such that the final buffer concentration was 0.05 M in phosphate buffer. The dipeptide concentrations were approximately 0.02 M. The pH was finally adjusted by adding the appropriate volume of phosphate buffers. Triplicate aliquots

(19) Kopple, K.; Ghazarian, H. G. *J. Org. Chem.* **1968**, *33*, 862.
(20) Kammera, T.; Lee, S. *Tetrahedron Lett.* **1979**, *46*, 4483.

of the solutions were sealed in glass tubing and heated in the oil bath. The tubes were cooled and opened, and the water was evaporated under a stream of air at 100 °C (oil bath). Although these conditions are rather drastic, they do not hydrolyze the dipeptide significantly. The dipeptides were separated by TLC after heating. This demonstrated that they were still present as the dipeptides. Only a very slight amount of hydrolysis occurs under these nearly neutral conditions. Dipeptides require strong acid (6 N HCl), high temperature (110 °C), and 22 h for hydrolysis.

Sample Derivatization. *N*-(Trifluoroacetyl)amino Acid Isopropyl Esters. The dipeptides were hydrolyzed to the amino acids with 6 N hydrochloric acid at 100 °C for 22 h, and the aqueous acid was removed as described above. The last traces of moisture were removed by azeotropic distillation with dichloromethane and evaporation under a stream of air. To each dried amino acid residue was added 2-propanol/HCl (4 N, 1 mL). The tubes were resealed and heated for 2 h at 100 °C. The excess 2-propanol was removed under a stream of air, dichloromethane was added, and the solvent was evaporated again. Derivatization was completed by adding 1-1.5 mL of trifluoroacetic anhydride in dichloromethane (30%). After the mixture had remained 20 min at room temperature, the excess reagent was removed by a stream of air. The residue was taken up in dichloromethane and transferred to small rubber-capped vials. There was no detectable racemization due to hydrolysis of peptide compounds or due to derivatizations.

Gas Chromatography.[21] Triplicate GC analyses were run on each sample using appropriate isothermal conditions at 140 °C. A stainless steel capillary column (150 ft × 0.02 in.) coated with an optically active mixed-phase consisting of 60% *N*-docosanoyl-L-valyl-*tert*-butylamide and 40% *N*-octadecanoyl-L-valyl-L-valylcyclohexyl ester was used. In all instances base line resolution was obtained for the D and L enantiomeric amino acid derivatives. The gas chromatograph used was an HP 5880A with electronic integration and a FID detector. The carrier gas was nitrogen.

Buffer Concentration Study. The ionic strengths of the samples were made identical by adding sodium chloride to the samples which were not of the highest buffer concentration (0.100 M). For example, the ionic strength of the 0.025 M phosphate buffer was made equal to that of the 0.100 M solution (μ = 0.1695) by adding 0.854 g of NaCl to 100 mL of the 0.025 M buffer.

(21) Smith, G. G.; Wonnacott, D. M. *Anal. Biochem.* **1980**, *109*, 414.

Acknowledgment. We express our sincere thanks and appreciation for a research grant from NASA, NSG-7038, and funds from the Research Office at Utah State University for the purchase of a JOEL FX 90Q FT NMR spectrometer.

Registry No. Val-Pro-OMe·HCl, 95500-47-3; Val-Pro (DKP), 2854-40-2; Gly-Val-OMe·HCl, 79638-93-0; Gly-Val (DKP), 16944-60-8; Val-Gly-OMe·HCl, 2421-57-0; Ala-Pro-OMe·HCl, 71067-42-0; Ala-Pro (DKP), 36357-32-1; Ile-Pro-OMe·HCl, 104465-31-8; Ile-Pro (DKP), 57089-60-8; Gly-Ala-OMe·HCl, 59095-76-0; Gly-Ala (DKP), 4526-77-6; Ala-Gly-OMe·HCl, 23404-09-3; Gly-Ile-OMe·HCl, 104465-32-9; Gly-Ile (DKP), 59652-63-0; Ile-Gly-OMe·HCl, 2421-59-2; Ala-Pro, 13485-59-1; Val-Pro, 20488-27-1; Ile-Pro, 37462-92-3; Ala-Gly, 687-69-4; Val-Gly, 686-43-1; Ile-Gly, 868-28-0; Gly-Ala, 3695-73-6; Gly-Val, 1963-21-9; Gly-Ile, 19461-38-2; Phe-Gly, 721-90-4; Gly-Leu, 869-19-2; Gly-Phe, 3321-03-7; Me-Gly-Ala, 53846-71-2; Me-Gly-Leu, 98951-55-4; Me-Gly-Phe, 17123-28-3; Me-Gly-Val, 98998-74-4; Me-Gly-Ile, 104465-33-0; Ac-Gly-Ala, 79806-70-5; Ac-Gly-Leu, 29852-55-9; Ac-Gly-Phe, 13716-72-8; β-Ala-Ala, 34322-87-7; β-Ala-Leu, 17136-25-3; β-Ala-Phe, 17136-28-6; β-Ala-Val, 17136-26-4; β-Ala-Ile, 104465-34-1.

Isomerization of the Hydridoalkylrhodium Complexes Formed on Oxidative Addition of Rhodium to Alkane C–H Bonds. Evidence for the Intermediacy of η^2-Alkane Complexes

Roy A. Periana and Robert G. Bergman*

Contribution from the Department of Chemistry, University of California, and Materials and Molecular Research Division, Lawrence Berkeley Laboratory, Berkeley, California 94720.
Received March 27, 1986

Abstract: The products of insertion of the coordinatively unsaturated fragment Cp*RhL (Cp* = η^5-C_5Me_5; L = PMe_3) into alkane C–H bonds are generated by irradiation of Cp*(L)RhH_2 or thermal decomposition of Cp*(L)Rh(neopentyl)(H), at temperatures below −30 °C in alkane solvents. The hydridoalkylrhodium products can be synthesized independently, also at low temperature, from the corresponding haloalkyl complexes by lithiation with *t*-BuLi followed by protonation. Alternatively, the highly nucleophilic complex Li[Cp*(L)RhH], prepared by deprotonation of Cp*(L)RhH_2, leads to the alkylhydridorhodium complexes upon treatment with alkyl tosylates R–OTs. When Cp*RhL is generated in mixtures of linear alkanes, only the products of insertion into primary C–H bonds are observed, even at −100 °C. The relative rate constants for the formation of these products correlate directly with the number of secondary C–H bonds in the alkane. This suggests that insertion occurs initially into all the C–H bonds of the alkane, but that even at very low temperatures the secondary insertion products rearrange quickly, and intramolecularly, to the primary products. We suggest that this rearrangement occurs through the intermediacy of η^2-C–H alkane complexes. Further studies provide support for this idea. The synthetic procedures summarized above can be used to prepare regiospecifically ^2H and ^{13}C labeled alkyl hydrides at low temperatures. Warming these complexes to temperatures above −80 °C in aromatic solvent reveals isotope rearrangements which confirm that the alkylhydridorhodium complexes are capable of interconverting with one another intramolecularly at rates competitive with or in some cases faster than they undergo reductive elimination. For example, rearrangement of the ^{13}C, ^2H-labeled ethylhydridorhodium and the (1-methylcyclopropyl)methylhydridorhodium complexes has been used to show that the Cp*(L)Rh fragment migrates and inserts most rapidly into the α position of the alkyl group followed by migration to the β and γ positions; reductive elimination is the slowest process which occurs. The kinetic isotope effect for oxidative addition of [Cp*(L)Rh] into the C–H bonds of hexane (determined by competition techniques) is small [(k_H/k_D) = 1.1 ± 0.1], whereas that for reductive elimination (obtained from directly measured rates) from the ethylhydridorhodium complex is both large and inverse [(k_H/k_D) = 0.5 ± 0.1]. These results provide further support for the existence of intermediate η^2-C–H alkane complexes.

Several organotransition-metal systems are now known in which intermolecular activation of alkane carbon–hydrogen bonds occurs. One important mode of reaction involves oxidative addition of relatively electron-rich, so-called "late" transition-metal complexes to C–H bonds in the alkane R–H, leading directly to isolable, or at least observable, insertion products R–M–H. Such alkane C–H oxidative addition has now been observed at iridium, rhodium, and rhenium centers.[1,2]

0002-7863/86/1508-7332$01.50/0